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Understanding the starch digestibility characteristics of Indian maize hybrids

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Maize (*Zea mays* L.) is one of important cereals of tropic and tropic countries. It serves as the raw material for starch production. Among starch types, ‘resistant start’ is considered more beneficial for human health. Hence, the genotype (of maize) gains significance in selection for commercial cultivation. However, nutritional information on starch digestibility of maize genotypes is scarce. In this context, we analyzed a set of 80 maize hybrids for carbohydrate profile (sugar, starch, amylose, amylopectin) and resistant starch content. The results revealed significant variation for carbohydrate profile and resistant starch content among diverse maize hybrids. Pratap QPM Hybrid was found to be the highest, followed by FCH 184, RMH 3591, NT 6240 and CO 1 in terms of sugar content. For total starch, Rasi 3033 hybrid proved the best genotype followed by L 333 and HQPM 7. The genotype LG 3271 exhibited highest amylose content followed by Bio 9544, P 3502 and DHM 119, whereas NMH 731 was found to possess highest amylopectin content followed by Janahit 5053 and KDMH 176. For resistant starch, LG 3271 was the best genotype followed by P3502, KH 2192 and HQPM 1. Amylose and resistant starch content showed highly significant positive correlation ($r = 0.550^{**}$), whereas highly significant negative correlation has been observed between amylopectin and resistant starch content ($r = -0.548^{**}$). The scanning electron micrographs of genotypes having the highest and the lowest values of resistant starch revealed substantial differences in the granular structure showing that starch granules are compactly packed in the LG 3271.

Keywords: Amylose, Amylopectin, Carbohydrate profile, Resistant starch, *Zea mays*

Maize (*Zea mays* L.) is one of the most widely distributed crops of the world cultivated in tropics, sub-tropics and temperate regions to almost all the conditions of irrigated to semiarid. It is, after wheat and rice, the most important cereal grain, providing nutrients for humans and animals and serving as a basic raw material for production of starch, oil, protein, alcoholic beverages, food sweeteners and, more recently, fuel. Globally, annual maize production in 2018 was reported to be 1147.62 MMT, of which Asia alone produces 359.20 MMT[1]. USA, China, Brazil, Argentina, Ukraine, Indonesia, India, Mexico, Romania and South Africa and are the top ten maize producing countries in the world. In India it the third most important cereal after rice and wheat cultivated in an area of about 9 m ha with an average yield of around 25 MMT of maize.

Nutritionally, maize grains contain starch (~70%), protein (7-13%) and small quantities of oil, sugar, vitamins and minerals. Starch is the most abundant storage polysaccharide, and is found in amyloplast of maize seeds[2]. Chemically, starches are polysaccharides, composed of a number of monosaccharides or glucose molecules linked together with α 1-4 as well as α 1-6 linkages. The starch consists of two main structural components, the amylose, which is essentially a linear polymer in which glucose residues are α 1-4 linked, and amylopectin, which is a larger branched molecule with α 1-4 and α 1-6 linkages[3]. In humans, starch is enzymatically digested, predominantly in the small intestine. Depending on the rate of release and absorption of glucose in the gastrointestinal tract, starch is classified into three groups: rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). RDS is the group of starches that can be rapidly hydrolyzed by digestive enzymes; SDS is the group that is digested at a relatively slower rate. However, a portion of starch, known as resistant starch (RS), resists enzymatic hydrolysis in the small intestine and passes to the large intestine for bacterial fermentation[4,5]. RS provides many health benefits to humans. When RS is used to replace rapidly digestible starch in food, it

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lowers the glycemic and insulin responses and reduces the risk of developing type II diabetes, obesity, and cardiovascular disease[6-8]. RS lowers calorie content of foods and enhances lipid oxidation, which reduces body fat and impacts body composition. Fermentation of RS in the colon promotes a healthy colon and reduces the risk of colon cancer[9,10].

Depending on their botanical origin, starches differ in their chemical structure, size and shape of their granules, and consequently in their functional and sensory properties[11,12]. Composition of maize starch is genetically controlled and normal maize starch consists of 25-30% amylose, and 70-75% amylopectin. However, the amylose extender mutant of maize increases the amylose content by up to 80% or more[13], whereas, waxy maize starch consists of almost 100% of amylopectin[14]. Because of its tightly packed structure, amylose is more resistant to digestion than amylopectin and the composition of amylose to amylopectin, therefore, may affect the digestibility of maize.

Maize is known to possess wide genetic variability with respect to its carbohydrate profile[15]. A large number of maize genotypes have been recommended for commercial cultivation in India. However, the nutritional information, particularly the starch digestibility characteristics of the above genotypes, is missing. Keeping in view the increasing utilization of maize for human consumption, here, we explored the maize genotypes of maize for starch digestibility characteristics.

Materials and Methods

The present study was conducted in the ICAR-Indian Institute of Maize Research, Pusa Campus, New Delhi. A set of 80 maize hybrids (Table 1), grown widely across India, particularly in the states of Andhra Pradesh, Telangana, Karnataka, Maharashtra, Bihar, some parts of Gujarat, Punjab, Himachal Pradesh and Jammu and Kashmir, were used in this study. The selected hybrids are high yielding at farmer's fields and are suitable to the agro-ecological conditions of their area of cultivation. The complete set of experimental hybrids was grown in Randomized Block Design (RBD) with three replications at experimental farm of Directorate of Maize Research (now ICAR-Indian Institute of Maize Research), Pusa Campus, New Delhi during *kharif* 2013. Selfed pollinated ears from each replication were harvested at maturity stage; seeds were shelled under shade and stored in dark at 4°C to prevent any loss of nutritional quality. The samples were oven-dried to reduce the moisture level in order to meet the accuracy of the results. Individual samples were ground into fine powder using a Cyclotech Mill (Model 1093, FOSS, Sweden), defatted using petroleum ether and finally kept in desiccators for analysis of various nutritional quality parameters.

Sugar content was determined by anthrone method[16]. Total starch, in a separate aliquot of the acetate solution, was similarly hydrolysed to D-glucose which was measured calorimetrically by glucose oxidase/peroxidase[17]. Amylose content was estimated using Megazyme Amylose/amylopectin Assay method K-AMYL[18]. The binding of lectin concanavalin A (Con A) to amylopectin offers an alternative and better approach to measurement of amylose, as compared to iodide binding assays. Amylopectin content was calculated by subtracting the amylose from total starch content. Resistant starch was estimated by using Megazyme Resistant Starch Assay K-RSTAR[19], whereby non-resistant starch was solubilised and hydrolyzed to D-glucose by treatment with pancreatic α-amylase and amyloglucosidase (AMG). The reaction was terminated by the addition of an equal volume of ethanol. RS was recovered as a pellet after centrifugation. The pellet was washed twice by suspension in ethanol (50% v/v), followed by centrifugation. Free liquid was removed by decantation. RS in the pellet was dissolved in 2 M KOH by vigorously stirring in an ice-water bath, kept over a magnetic stirrer. Acetate buffer was used to neutralize the solution and the starch was quantitatively hydrolysed to glucose with AMG. D-Glucose was measured with glucose oxidase/ peroxidase reagent (GOPOD) and this was taken as a measure of the RS content of the sample.

The surface topography of product samples was observed by scanning electron microscope (SEM)[20]. Samples having the highest (LG3271) and lowest (FCH184) resistant starch percentage were analyzed at 3.00 kx magnification. Dried, seed samples were mounted on an aluminium stub using double-sided tape and coated with a thin film of gold. The samples were examined at an accelerating voltage of 20 kV.

Statistical analysis

Descriptive statistics and analysis of variance (ANOVA), correlation between biochemical traits was done using Statistical Analysis Software (SAS 9.2 English). A Pearson Correlation Coefficient $|r|$

Table 1 — Detailed information of Experimental maize hybrids along with the organization responsible for their development

S. No.	Hybrids	Organization	S. No.	Hybrids	Organization
1	KDMH 4086	Krishidhan Seed Pvt. Ltd.	41	Nirmal 3662	Nirmal Seeds Pvt. Ltd.
2	EH 1974	MPUA & T, Udaipur	42	CMH 08 -292	TNAU,Coimbatore
3	GEO9099	GEO Biotech India Pvt. Ltd.	43	KMH 25 K 60	Kaveri Seed Comp. Ltd.
4	HQPM5	HAU, Karnal	44	NMH 731	Nuziveedu Seeds Ltd.
5	Rasi 3033	Rasi Seeds Pvt. Ltd.	45	CP 999	CP Seeds Pvt. Ltd.
6	NAH 2049	ZARS,VC Farms, Mandya	46	Siri 4546	Siri Seeds Pvt. Ltd.
7	P 3501	Pioneer Overseas Corporation	47	RMH 972	Rasi Seeds Pvt. Ltd.
8	FCH184	Foliage Crop Solution Pvt. Ltd.	48	MHM 2	BHU, Varanasi
9	Nirmal 27	Nirmal Seeds Pvt. Ltd.	49	CP 828	CP Seeds Pvt. Ltd.
10	PMH 3	PAU, Ludiana	50	NK 6217	Syngenta India Ltd.
11	DHM 119	MRC, ANGRAU, Hyderabad	51	Bio 9544	Bioseed Research India Pvt. Ltd.
12	GEO PREMIUM DIAMOND	GEO Biotech India Pvt. Ltd.	52	Capital	Yaaganti Seeds Pvt. Ltd.
13	Janahit	Godrej Seeds & Genetics Ltd.	53	P 3502	Pioneer Overseas Corporation
14	NT 7303	Syngenta India Ltd.	54	LG 32-71	Bisco Bioscience Crop Pvt. Ltd.
15	SAFAL X 1	Safal Seeds & Biotech Ltd. Jalna	55	KMH 218 Plus	Kaveri Seed Comp. Ltd.
16	Bio 719	Bioseed Research India Pvt. Ltd.	56	FCH 85	Foliage Crop Solution Pvt. Ltd.
17	DKC 9125	Monsanto India Pvt. Ltd.	57	Super GA 105	Godrej Seeds & Genetics Ltd.
18	FCH 38	Foliage Crop Solution Pvt. Ltd.	58	GEO 2101	GEO Biotech. India Pvt. Ltd.
19	NMH 1247	Nuziveedu Seeds Ltd.	59	PMH 1	PAU, Ludiana
20	KH 115-08-01	Kanchan Ganga Seed Pvt. Ltd.	60	RMH 932	Rasi Seeds Pvt. Ltd.
21	DKC 7074	Monsanto India Pvt. Ltd.	61	POLO	Kanchan Ganga Seed Pvt. Ltd.
22	CMH 08-282	TNAU, Coimbatore	62	NT 6240	Syngenta India Ltd.
23	PMH 4	PAU, Ludhiana	63	Geo diamond	GEO Biotech. India Pvt. Ltd.
24	KH 2192	Kanchan Ganga Seed Pvt. Ltd.	64	FMH 11195	Foliage Crop Solution Pvt. Ltd.
25	Dada	Yaaganti Seeds Pvt. Ltd.	65	KDMH 176	Krishidhan Seed Pvt.Ltd.
26	Pratap QPM Hybrid 1	MPUA & T, Udaipur	66	DKC 9106	Monsanto India Pvt. Ltd.
27	HM 8	HAU, Karnal	67	PAC 753	Advanta India Ltd.
28	KDMH 17	Krishidhan Seed Pvt. Ltd.	68	DHM 113	MRC, ANGRAU, Hyderabad
29	PAC 745	Advanta India Ltd.	69	NMH 920	Nuziveedu Seeds Ltd.
30	KMH 2589	Kaveri Seed Comp. Ltd.	70	L 4959	Yaaganti Seeds Pvt. Ltd.
31	PAC 740	Advanta India Ltd.	71	ARJUN	Safal Seeds &Biotech Ltd. Jalna
32	EC 3161	MPUA & T, Udaipur	72	LG 3281	Bisco Bioscience Crop Pvt. Ltd.
33	KMH 22168	AICRP Maize, Kolhapur	73	HM 12	HAU, Karnal
34	L 333	Yaaganti Seeds Pvt. Ltd.	74	Co 1	TNAU, Coimbatore
35	SAFAL X 2	Safal Seeds & Biotech Ltd. Jalna	75	Nirmal 3493	Nirmal Seeds Pvt. Ltd.
36	DHM 117	MRC, ANGRAU, Hyderabad	76	Siri 4527	Siri Seeds Pvt. Ltd.
37	RMH 3591	Rasi Seeds Pvt. Ltd.	77	CMH 08-287	TNAU, Coimbatore
38	30 B 07	Pioneer Overseas Corporation	78	HQPM 1	HAU, Karnal
39	KMH 3712	Kaveri Seed Comp. Ltd.	79	TX 369	Bioseed Research India Pvt. Ltd.
40	NMH 1277	Nuziveedu Seeds Ltd.	80	HQPM 7	HAU, Karnal

among 80 maize hybrids was calculated by taking Prob> | r | under (Null Hypothesis) H_0: Rho = 0 by Statistical Analysis Software.

Results and Discussion

The average carbohydrate profile of the experimental hybrids showed three-fourth proportion of amylopectin, followed by amylose, sugar and smaller quantity of Resistant Starch (Fig. 1). The values of each carbohydrate are presented in the form of percentage. Figure 2 shows the frequency distribution of different carbohydrates in experimental hybrids, starting from smallest to largest values. The frequency distributions are skewed towards larger amylopectin and smaller amylose values (Fig. 2A and 2B). The amylose and amylopectin content were found to be negatively correlated (Fig. 1B). Sugar content of experimental hybrids does not follow a normal distribution (Fig. 2C), whereas resistant starch content shows a normal bell-shaped distribution. A significant variation for carbohydrate content was found in the experimental genotypes (Fig. 2). Sugar content varied from 3.64% (NMH 1277) to 5.59% (Pratap QPM Hybrid) with mean value of 4.44%.

Most of the genotypes exhibited sugar content between 4 and 4.5%, whereas only a few genotypes were found to possess more than 5% free sugar content in their mature kernels. Sugar is an important component which is mostly present as glucose, sucrose and fructose, and renders sweetness to the maize kernel. Higher sugars are present in maturing maize kernels before its further conversion to the reserve carbohydrate in the mature seeds. The conversion rate of sugars to starch depends upon the genetic makeup[21], resulting in different sugar contents in the experimental genotypes.

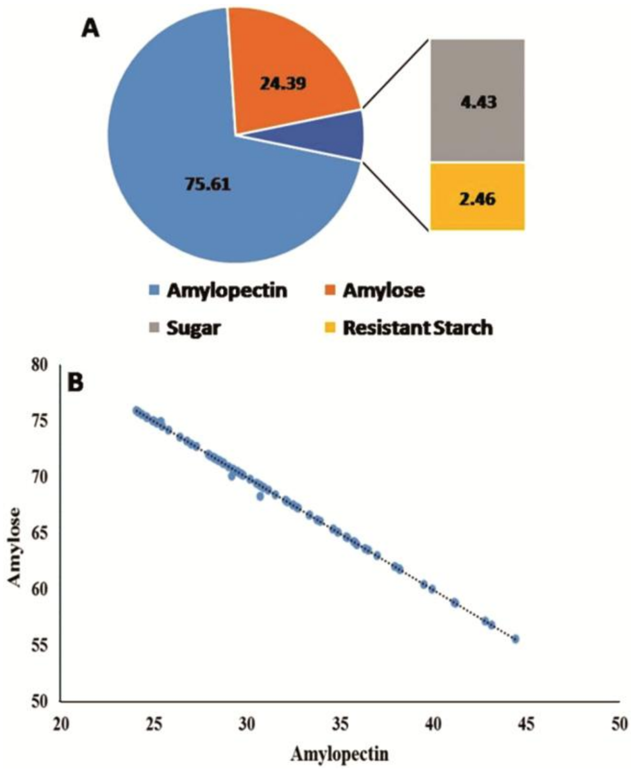

Fig. 1 — (A) Average carbohydrate profile of maize hybrids. The mean values of amylopectin, amylose, sugar and Resistant Starch (in percentage) of the experimental maize genotypes are shown with respect to their proportion; and (B) Correlation between amylose and amylopectin content of maize hybrids. [The amylose and amylopectin content (in percentage) of the maize genotypes displayed a perfect negative correlation]

Starch is the reserve carbohydrate of maize kernel and is the major source of nourishment for humans and animals. A significant variation from 67.89% (CMH 08-287) to 75.94% (Rasi 3033) was observed in starch content of experimental hybrids. Starch is, quantitatively, the most important carbohydrate in the diet of most humans and their principal source of dietary energy. Although starch usually accounts for 60% of energy intake in developing countries, but, its consumption for human food is continuously declining in western world, where adult consumption ranges between 120 to 150 g of starch daily. Variation in the composition of cereal starch, in terms of the amylose to amylopectin ratio, is governed by the genome and its genetic potential to undergo mutations[19]. Maize has been found to possess 68-73% starch[18]. However, the extractable starch content was found to be little lower[22,23]. Variation was observed in the starch profile of experimental hybrids. Amylose and amylopectin varied from 24.08% (NMH 731) to 44.42% (LG 3271), and 55.06% (LG 3271) to 76.63% (NMH 731), respectively.

Amylose is helical polymer made of α-D-glucose units, bonded to each other through α (1→4) glycosidic bonds and owing to its linear structure it occupies less space and, therefore, is tightly packed in starch granules. Normal maize starch consists of 15-30% amylose, depending on the botanical origin, degree of maturity, growing conditions, and the method used for determination[24,25] , whereas high-amylose starch usually consists of more than 50% amylose[13,26]. One of the maize mutants, amylose-extender (*ae*) mutant, produces starch with a much larger amylose-content and amylopectin with significantly longer branch-chains than the normal maize starch[27-31]. Because the long linear chains of

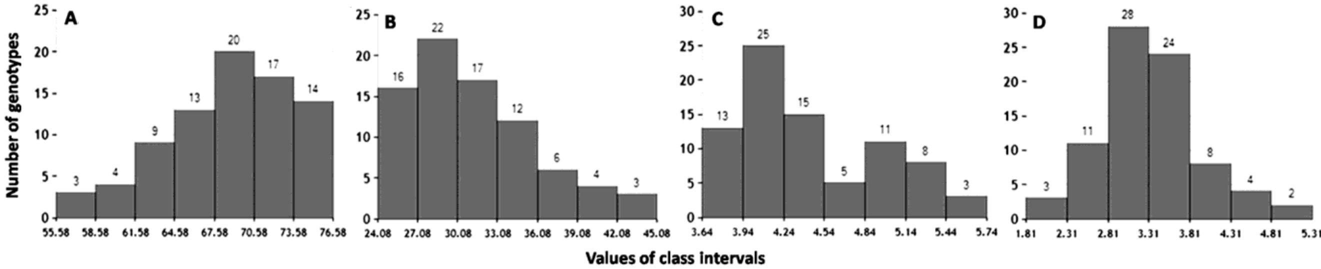

Fig. 2 — Frequency distribution diagrams of (A) Amylopectin; (B) Amylose; (C) Sugar; and (D) Resistant starch. [The content of each carbohydrate was classified into seven class intervals. The number of genotypes representing a particular class interval are depicted on top of each interval]

amylose more readily crystallize than amylopectin (which has short, highly branched chains), high-amylose starch is more resistant to digestion. To study the resistant starch content of experimental hybrids, samples were subjected to *in vitro* enzymatic digestion and the resistant starch maize was isolated and evaluated. To validate the results, samples showing highest and lowest values of resistant starch were analyzed through scanning electron microscopy (SEM). Results revealed a significant variation for resistant starch ranges from 1.81% (FCH184) to 5.12% (LG 32-71).

Starch digestion is initiated in the mouth by the action of salivary α-amylase which continues in the smaller intestine, under the action of pancreatic α-amylase and specific brush border hydrolases[32]. The overall contribution of these amylases is that most of the starches are digested and absorbed in the duodenum in a matter of few minutes, as is clearly evidenced by the swift rise of blood glucose concentration following consumption of refined starchy foods[33]. Not all starch that is eaten are rapidly digested. A portion of starch, referred to as resistant starch (RS), cannot be digested and absorbed in the small intestine and is passed to the large intestine for bacterial fermentation[2]. RS is classified into four types. Type I RS is starch that is entrapped in plant tissue and not susceptible to enzyme hydrolysis. Type II RS consists of native raw starch granules having the B-type polymorphism, such as potato, wrinkle pea, and high-amylose maize starches, which are resistant to enzyme hydrolysis. Type III RS is retrograded amylose, and Type IV RS is chemically modified starch[2,34]. Identification of resistant starch or slowly digestible starch maize cultivars will immensely help general population, particularly diabetics in managing their nutritional requirements. Studies have suggested that consumption of RS made from high-amylose maize starch, brings a wide range of health benefits, such as lowering the glycemic index and promoting colon health[35]. Resistant starch has many health benefits as prevention of colonic cancer[35,36], as a pre-biotic agent[37], inhibition of fat accumulation[38] and absorption of minerals[39,40]. The RS content in native starch of various ZP genotypes was very low[41] (<1.61%). An exceptionally high degree of resistance to amylolytic enzymes was displayed by starch of *ae*-VII hybrid of maize[42]. High-amylose maize starches consist of a large proportion of RS (11.5 to 43.2%) determined using AOAC method for total dietary fiber[12,43].

Correlation between carbohydrate profile and resistant starch components of maize hybrids was analyzed (Table 2). Amylose and resistant starch content showed significant positive correlation (r = 0.550**), whereas highly significant negative correlation has been observed between amylopectin and resistant starch content (r = –0.548**). Sugar and amylose showed no correlation (r = 0.044). Sivert & Pomeranz[42] reported positive correlation between amylose and RS. They have reported that amylo-maize VII contain 70% amylose and 21.3% RS. A higher content of amylose lowers the digestibility of starch due to positive correlation between amylose content and formation of RS[35]. The amylopectin is a much larger molecule than amylose; therefore, due to its larger surface area per molecule, it is a preferable substrate for amylolytic enzymolysis. Sievert & Pomeranz[44] observed that peas with 33% amylose showed 10.5% of RS and potatoes with 20% amylose showed 4.4% of RS. To validate our results, grains of the highest and lowest resistant starch genotypes were viewed under scanning electron microscope (Fig. 3). Micrograph of LG 32-71 showed larger granules, more compactness, attributing to small surface area thus decreasing the extent of enzyme hydrolysis, whereas grains of FCH 184 hybrid contain smaller

Table 2 — Correlation between carbohydrate components and resistant starch of maize hybrids

Variable	Sugar	Starch	Amylose	Amylopectin	RS
Sugar	-	–0.112	0.044	–0.013	–0.011
Starch		-	–0.160	0.160	–0.185
Amylose			-	–1.000**	0.550**
Amylopectin				-	–0.548**
RS					-

[Pearson Correlation Coefficients, N = 80; Prob > |r| under H0: Rho=0. The correlation between carbohydrate profile and resistant starch was calculated using Statistical Analysis Software (SAS 9.2 English). ** indicates that the correlation is significant at the 0.01 level (2-tailed)]

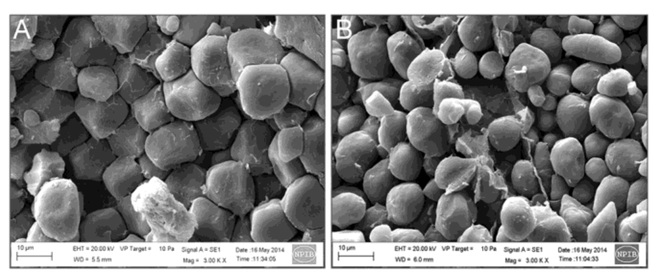

Fig. 3 — Scanning electron micrograph of (A) LG 3271 maize hybrid; and (B) FCH 184 maize hybrid. [The structures of starch granules of the genotypes displayed highest (LG 3271) and lowest (FCH 184) amounts of resistant starch were analyzed by Scanning electron microscopy]

granules, less compactness which attributed to large surface area, thus increasing the extent of enzyme hydrolysis. The surface characteristics of the starch granules have been observed to influence their enzymatic digestion. Pin holes, equatorial grooves and small nodules have an impact on the entry of the amylases to digestion[45,46]. Other starches such as potato and high amylose starches have smoother surface and fewer pits or pores which can explain the resistance of these starches to amylases[47,48].

Conclusion

From the above results, it can be concluded that a large variability exists in the carbohydrate profile and resistant starch content among the experimental hybrids. Genotypes, namely LG 3271, P3502, KH 2192 and HQPM 1 have been found to be promising for resistant starch. Amylose and resistant starch content were found positively correlated ($r = 0.550^{**}$), whereas highly significant negative correlation has been observed between amylopectin and resistant starch content ($r = 0.548^{**}$). It was also observed that the experimental genotypes are skewed towards higher amylopectin and lower amylose content. Resistant starch showed a normal bell-shaped distribution. Scanning electron micrographs revealed the structural differences between tightly-packed and loosely-packed starch genotypes, contrasting in Resistant Starch. Hence, the resistance of starch digestibility is linked to the structure of starch granules in maize.

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Conflict of interest

Authors declare no conflict of interests.

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